FORAGING, SPATIAL DISTRIBUTION, AND CONTROL OF THE PHARAOH ANT, *MONOMORIUM PHARAONIS* (L.)

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1996

ACKNOWLEDGEMENTS

I wish to thank Dr. David F. Williams for all the support he provided me over the past five years. He served a dual role in my life, not only was he the chairman of my committee, but he also served as my supervisor at the USDA, ARS, Medical and Veterinary Entomology Research Lab. This role sometimes challenged his good naturedness, but somehow we always managed to get things done. I know observers thought I treated him with "no respect," but it is with the deepest respect and appreciation that I thank him.

To the rest of my committee, J. Allen, P. Koehler, R. Patterson, S. Porter, and J. Reiskind, I also extend my thanks. Serving on my committee was a challenge, especially during my qualifying exam. I should imagine they were a little apprehensive when asking questions to a woman who was eight months pregnant and had a history of a premature birth. I would particularly like to thank P. Koehler for the opportunity to prepare a pictorial ant key and to present extension talks on ant control and identification. Not only were they challenging and educational, but they were instrumental in the successful procurement of a

professional position.

My friends and coworkers at the USDA have made the completion of my research possible, especially during the last two months of my pregnancy with Virginia. I thank Eileen Helms, Greg Knue, Darrell Hall, Gary Worth, Lloyd Davis, and Terry Krueger for their help, advice, friendship, and inspiration. David Oi functioned as my statistical consultant and role model, and often convinced me to continue my pursuit of this degree. To Margaret Martin I also extend my gratitude. She often helped by entertaining my three year old when things got tough.

I would like to thank Myrna Litchfield, for the advice she imparted when I was having difficulty finding time to study. The advice she gave was simple, "Just get up early and study." I heeded her advice and was able to complete my classes and pass my qualifying exam.

And most importantly I must confess this would all have been impossible if it had not been for the love and understanding of my husband, Pat Parkman. He was my pillar. He maintained the household and cared for our two children, Kelsey and Virginia, so I would have time for research and writing. Someway, somehow, I will make it all up to him.

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Abstract of Dissertation Presented to the Graduate School of
the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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May 1996

Chairman: David F. Williams
Major Department: Entomology and Nematology

The Pharaoh ant, *Monomorium pharaonis* (L.), is a pervasive ant found in urban structures. Calco® red and blue dyes were mixed in either peanut oil or peanut butter at 0.25 - 2% to mark Pharaoh ants' postpharyngeal glands and crops. Neither mixture caused an increase in mortality or was repellent when compared to the control in the laboratory. Dyes mixed in peanut butter at 2% (w:w) were used to determine foraging behavior at a military officer's quarters (7,841 m^2 of floor space) in Jacksonville, FL. Mean (± SD) dispersal distance was 16.2 ± 9.6 m and the maximum distance 45 m. More ants were found indoors per card (128.4 ± 13.0, mean ± SEM) than outdoors (64.0 ± 18.4) probably indicating the majority of ants nested indoors.

Pharaoh ant colonies introduced into eight buildings ($7.4\ m^2$ of floor space each) initially budded into a mean of 7.5 colonies distributed over a mean of 3.5 walls. This did not prevent fire ants, *Solenopsis invicta* Buren, from entering the buildings and reducing the Pharaoh ant populations (colony size and number). Fire ants caused the formation of small, ephemeral nests on the panelling facing the interior of the room. Pharaoh ants were found to nest outdoors in Gainesville, FL; this had never been observed prior to this study. Fire ants also limited the duration of Pharaoh ant nests in the soil. Pharaoh ants attempted to repel fire ants by presumably spraying repellent while flagging their gasters. More Pharaoh ant nests were found in the attic than in the walls; most of the nests (51.4%) were found resting between the paper of the insulation and panelling.

Pharaoh ant colonies of different sizes ranging from about 8,000 to 26,000 workers were observed to determine the effects of starvation (2 and 96 h) on foraging activity. Colony size and starvation increased the number of foragers at a bait. The percent of workers foraging decreased as colony size increased.

Pyriproxyfen, a juvenile hormone analog, applied to the exterior of a military officer's quarters controlled natural infestations of Pharaoh ants. The bait formulation included a peanut oil attractant applied to a corn grit carrier.

Difficulties conducting field tests for Pharaoh ant control in a large structure with separate wings are discussed.

CHAPTER 1
INTRODUCTION

The Pharaoh ant, *Monomorium pharaonis* (L.), is a small tramp species about 2 mm long which ranges in appearance from yellow to light red. Identifying features include a 2-segmented waist, 12-segmented antennae with a 3-segmented club, and a sting; also its color is uniform except for the apical portion of the gaster which is darker (Haack and Granovsky 1990, Hedges 1992, Smith and Whitman 1992, Vail et al. 1994). This ant was first identified as *Formica pharaonis* by Linnaeus in 1758 and was given at least six other specific names (Bolton 1987) before receiving its current name (Mayr 1882). It was thought that this species originated in South America (Arnold 1916) or the Afrotropical region (Bernard 1952); however, Bolton (1987) suggested that its origin is India, an idea first put forth by Emery (1922) and Wilson and Taylor (1987). Because it has been introduced to many parts of the world through international trade, its place of origin may never be determined. *M. pharaonis* is pervasive throughout Europe (Eichler 1974, Edwards and Baker 1981, Richter et al. 1981). Smith (1965) thought it was so common a pest as to state "the Pharaoh ant is probably found in every town or city of commercial importance in the United States"(p. 34). The Pharaoh ant is a major pest ant in Florida (Bieman & Wojcik 1990, Klotz et al. 1995a, 1995b).

Tramp species are typically polygynous (colonies contain two or more egg-laying queens), synanthropic (live with humans in their structures), and often distributed by commerce (Holldobler and Wilson 1990). The Pharaoh ant's unusual reproductive strategy undoubtedly contributes to its pest status. Like other typical tramp ants, this species does not require mating flights for sexual reproduction because they reproduce by budding; to start a new colony, workers carry their brood to another location. This movement may or may not be accompanied by queens (Peacock et al. 1955). Workers then rear sexual brood and adult reproductives to perpetuate the colony. A colony contains the immature stages or brood (eggs, larvae, prepupae, and pupae) and adults (sterile workers, mated and unmated females, and, rarely, males). Alvares et al. (1993) summarized development times from Peacock (1950), Peacock et al. (1950), Petersen-Braun (1973), Kretzchmar (1973) and Berndt and Eichler (1987). They found the egg stage required 6-11 d for development, the larval stage required 7-18 d, prepupae required 2-7 d, and pupae required 7-12 d. The total development time from egg to worker of 22-48 d. Total development time for immature stages of a Brazilian population was found to be 25-54 d (Alvares et al. 1993).

Alvares et al. (1993) also confirmed existence of 3 larval instars in the Brazilian population.

Budding makes the Pharaoh ant difficult to control because there are often groups of related nests instead of only one. Factors that influence budding include changes in the environment such as temperature and food and water resources, or overcrowding (Edwards 1986). The presence of insecticides is also suspected to cause budding (Green et al. 1954).

Nest location can also make control difficult. Nests are unstructured and usually located in inaccessible or difficult to reach areas for the pest control operator, including interior wall voids, areas under or behind window sills, toilets, sinks, switch plates, lights, etc. Edwards (1986) suggests that any warm area with high humidity can serve as a nest site. We have noticed at several hotels, motels and hospitals in north Florida that voids in aluminum window and door frames are popular nest sites. Although there are reports that suggest the Pharaoh ant nests outdoors in southern Florida (Creighton 1950, Krombein et al. 1979), in temperate regions it usually nests indoors [with an outdoor garbage dump noted as an exception (Kohn and Vlcek 1984, 1986)]. In the Tampa, Florida, area, nests were located in dead tree horns that extended over houses; foraging workers were found following fences (pers. obs.). This species has been reported as living outdoors in Victoria county, Texas

(Mitchell and Pierce 1912), about 25°N latitude which is closer to the equator than Gainesville, Florida. Outdoor nest locations were in woods under bark of logs and live trees. In north central Florida (Gainesville), Pharaoh ants have been observed foraging both indoors and along the outdoor periphery of the building, but no nests have been detected outdoors.

Pharaoh ant foraging has been described by Sudd (1957, 1960), Haack and Vinson (1990) and Fourcassie and Deneubourg (1994). The following description is from Sudd. To initiate their search for a food source, scouts followed routes along cracks or edges of structures, a phenomenon described by Klotz and Reid (1992) as guideline orientation. As the scouts approach a food source, the trails branch. The return trips to the nest were more direct than the outgoing trips possibly indicating workers use visual orientation as well as odor trails. Workers in the nest were recruited by stimulating them to search for food. This stimulation was accomplished by workers returning to and rushing about the nest without any apparent stimulatory contact with other workers. Food location was identified by following the trail pheromone, faranal, which was laid by the scouts. Foragers also reinforced the trail pheromone. Faranal ($C_{17}H_{32}$) is a terpenoid produced by the Dufour's gland (Ritter et al. 1977a) which is located in the gaster.

Fourcassie and Deneubourg (1994) describe the Pharaoh ant exploratory behavior when colonies under different starvation levels were given access to marked (previously exposed to Pharaoh ants) and unmarked areas. Number of ants exploring increased with starvation. Number of outbound foragers was greatest for starved colonies on unmarked areas followed by fed colonies on unmarked areas followed by starved colonies on marked areas followed by fed colonies on marked areas. Whether the area was marked or not was more important than the starvation level of the colony. When colonies were starved for 10 d, almost all of the colony joined in the exploratory foraging behavior. Trunk trails at 30-50°, 110-120°, 230-240° and 260-270° developed within 15 min of the exploratory behavior and acounted for 60-90% of the foraging activity over a 2 d period. The rapid exploratory response of many foragers to a new area could aid in the preemption of other species (Fowler 1993, Fowler et al. 1993). Fourcassie and Deneubourg (1994) observed a positive phototropism or an increase in activity with illumination of the arena. This finding was corroborated in our laboratory when Pharaoh ants followed a light path of a flashlight as it moved around a tray containing these ants (pers. obs.).

Pharaoh ant control currently emphasizes the use of toxic baits, though in the past 100 years, control of Pharaoh ants has included the use of insecticidal sprays and baits. Insecticides evaluated as residual sprays or dusts include pyrethrum (Lintner 1895), chlordane, DDT and benzene hexachloride (Rogers and Herrick 1953), dieldrin (Morgan and

Price 1954, Papworth 1958), and aprocarb (Kane 1967). Active ingredients in baits have included boric acid (Riley 1889, Newton 1980), *Bacillus thuringiensis* (Vankova et al. 1975), chemosterilants (Berndt et al. 1972), sulfluramid (Williams and Vail 1994, Oi et al. 1994), hydramethylnon (Oi et al 1994) and the juvenile hormone analogs methoprene and fenoxycarb (Edwards 1985, Williams 1990, Wilson and Booth 1981, Williams and Vail 1993, 1994). Insecticidal sprays and dusts are often ineffective because they affect only a small percentage of the workers that forage (Williams 1990). They may also cause budding which amplifies the problem. Multiple nests within a relatively small area also reduce the effectiveness of sprays. Residual sprays may be used to reduce foragers in sensitive areas such as surgery and neonatal care units in hospitals, but the use of sprays in hospitals is severely restricted in Florida. Unlike sprays which usually do not contact a nest, a toxic bait can be transported to the nest by foragers which feed on the bait and then distribute the toxin throughout the colony by trophallaxis.

Stomach poisons or insect growth regulators (IGRs) are the active ingredients in toxic baits. Stomach poisons used in some commercially available baits include Maxforce® Pharaoh Ant Killer bait, Combat® SuperBait Advanced Formula Ant Control, Maxforce® Professional Insect Control® Ant Killer Granular Bait, and Combat® Outdoor Ant Killing Granules (hydramethylnon, Clorox Co., Oakland, CA); Raid Max Ant Bait (sulfluramid, S. C. Johnson, Racine, WI); FluorGuard Ant Control Baits (sulfluramid, FMC Corp., Philadelphia, PA); Pro-Control®, Dual Choice (sulfluramid, Micro-Gen Equipment Corp., San Antonio, TX); Drax® Ant Kil PF, Drax® Ant Kil Gel, CB® Drax® Ant Control Systems (orthoboric acid, Waterbury Co. Inc., Waterbury, CT); and Terro® Ant Killer II (sodium tetraborate decahydrate [borax], Senoret Chemical Co., St. Louis, MO). An early recommendation for Pharaoh ant control was the use of borax and sugar dissolved in boiling water and placed on broken crockery (Riley 1889). In the past, our use of boric acid in laboratory evaluations gave variable results, but recent research of a 1% boric acid-sugar water solution has been effective when given to colonies continuously (Oi et al. 1995). Pest control operators (PCOs) use the boric acid bait because they can choose the attractant. Foraging workers are attracted to many compounds and the PCOs mix the boric acid with their choice of attractive ingredient or combination of ingredients.

The only insect growth regulator that was registered for Pharaoh ant control in the past was methoprene, marketed under the name of Pharorid® (Zoecon Corp., Dallas, TX). IGRs are much slower acting than stomach poisons because typically only the queen and brood are affected. Methoprene induces sterility in queens and disrupts the brood stages (Edwards 1975b). Workers are unaffected and are still evident for weeks or months after treatment (Edwards 1975a, 1982, Edwards and Clark 1978). Because the maximum worker life span at 27°C is estimated to be 9-10 weeks (Peacock and Baxter 1950), they may be present long after queens have ceased egg production and the brood has died. The advantage of using insect growth regulators is that they are more likely to be distributed throughout the entire colony because they do not adversely affect workers. Also IGRs are more acceptable to the consumer since they are considered safer compounds. The oral LD_{50} of methoprene to rats is >34 g/kg (Ware 1983).

Stomach poisons work relatively fast compared to IGRs, i.e., worker numbers are reduced in a few days and complete colony elimination can occur in a few days to a few weeks. However, stomach poisons may work too quickly, thereby eliminating the worker force before the insecticide can be distributed to the entire colony. Relatively few workers and brood need to survive to perpetuate the colony. Peacock et al. (1955) have reported just 100 workers and 50 pieces of brood can initiate a successful colony. Vail (unpublished) has found that 5 workers and 30 eggs, 19 larvae and 3 pupae can start a new colony.

Pharaoh ant foraging is used as an indicator of colony presence; without foragers these cryptic nests would go unnoticed. Therefore, more information is needed about Pharaoh ant foraging behavior and what this behavior can reveal about the colony or nest. In this study the four objectives on foraging behavior were to do the following:

1) observe foraging in a large military officer's quarters;
2) identify nest locations in and around small buildings and determine if presence of foragers indicated a nearby nest;
3) determine if starvation level and colony size affected foraging activity; and
4) evaluate the effectiveness of an insect growth regulator, pyriproxyfen, against a natural infestation of Pharaoh ants.

CHAPTER 2
DEVELOPMENT AND USE OF A MARKING TECHNIQUE FOR MEASURING PHARAOH ANT FORAGING IN A LARGE STRUCTURE

Introduction

In July, 1990, a Pharaoh ant infestation was located at the Bachelor Officer's Quarters (BOQ), Jacksonville Naval Air Station, Jacksonville, FL. Fenoxycarb (Maag Agrochemicals now part of Ciba-Geigy, Greensboro, NC) an insect growth regulator, was evaluated for control of the Pharaoh ants in this approximately 7841 m^2 facility (Williams and Vail 1994). Ants were absent from monitoring cards at 6 weeks after treatment in the 0.5% fenoxycarb-treated wing and were not found again until week 24, indicating the IGR gave at least 18 weeks of control. The presence of ants in the 0.5% fenoxycarb-treatment suggested that foragers from the south wing were entering the west wing (0.5% fenoxycarb-treament) where a vending machine area was located. To determine if Pharaoh ants could travel that distance for food, a foraging study was initiated. Sudd (1957, 1960) reported on foraging, recruitment and communication of Pharaoh ants. Haack (1987) and Haack et al. (1995) studied food flow in small colonies of the Pharaoh ant, but neither observed foraging in a building the size of the BOQ with such a large Pharaoh ant infestation. The objective of this study was to develop, in the laboratory, two dyes in food baits that could mark Pharaoh ants for a minimum of four days. This marking method would then be used at the BOQ to observe foraging (number of ants at bait, percentage of ants containing dye, and distance from dyed bait) in the Pharaoh ant both indoors and outdoors.

Materials and Methods

Development of a Marking Method

Oil soluble dyes (Calco® red and blue) in unprocessed peanut oil. Small colonies of Pharaoh ants were formed by removing one nest from a large colony containing several nests. The Pharaoh ant colonies were maintained at the Medical and Veterinary Entomology Research Laboratory in Gainesville, Florida. Each nest contained at least 3.5 g of brood, workers and about 100 queens. Nests were placed in a plastic shoebox which had Teflon® coating (Teflon® 30-B, Dupont Polymers, Wilmington, DE) on inner walls to prevent the ants from escaping. Foragers were taken from the large colony by brushing foragers from the food cup and water tube and by making several sweeps across the bottom of the large tray with an index card and tapping workers into the shoebox. A water dish consisting of a small plastic cup (15 ml) containing a cotton ball was moistened daily. Food (house flies, honey-agar and hardboiled egg) was provided twice a week. Twenty-four hours prior to dye introduction, the food was removed.

Dyes, Calco® oil red N-1700 and Calco® oil blue zv (Pylam Products Co., Inc., Garden City, NY) were formulated in unprocessed peanut oil (Stevens Industries, Dawson, GA) at 0.25, 0.5, 1, and 2% (w:v). Dyes were introduced into a colony using ten 100 microliter pipettes. Once the pipette was filled with the dye solution, one end was dipped into melted paraffin. The hardened paraffin prevented the dye from leaking and allowed the ants to chew throught it if the other end became clogged. Two colonies received each dye. Two colonies received unprocessed, nondyed peanut oil in the same manner as the dyed peanut oil. Oils were removed 24 h after introduction and the colonies were given their regular diet. The percentage of oil consumed and the number of dead workers were recorded. To determine the percentage of foragers containing dye, workers were tapped from the water dish (to ensure they were foragers) onto a sheet of white paper. The paper was folded, and the ants were crushed with a heavy metal roller. The first 20 ants from the right side of the paper were circled with a pencil to prevent any bias in selecting ants. The ants were then brushed away and the circles noted to contain dye or not. Workers were observed at day 1,2,3, and 4 after treatment, and irregularly thereafter.

Five queens per colony were crushed on day 4 to determine if they contained dye. Dye was observed in the postpharyngeal gland and thereafter; this gland was observed in the queens to determine if queens contained dye. Comments were also recorded on the stages of dyed brood and the ease of detecting the dye.

This method, of feeding ants dyed food and crushing them to determine if they contained dye, was modified from Lofgren et al. (1961). Lofgren used Calco® oil red N-1700 and Calco® oil blue zv in soybean oil, but we chose to use unprocessed peanut oil because it was preferred by Pharaoh ants (Williams 1990).

Oil soluble dyes in peanut butter. Because the method with the melted paraffin would be difficult to use outside of the laboratory (the dye could spill, a hot plate is required to melt the paraffin, etc.) a similar method was used as described above except that peanut butter was substituted for unprocessed peanut oil. Calco® oil red N-1700 and Calco® oil blue zv dye were incorporated into creamy peanut butter at 1 and 2% (w/w). Each treatment was replicated three times. Colonies consisted of 3.5 g brood and about 100 queens, but because there was a lack of colonies that fit this criterion, only two control colonies were used. Peanut butter was removed 24 h after introduction and the regular diet provided thereafter. The percentage of the peanut butter consumed was determined by weight. The number dead, and the percentage of foragers and queens containing dye were calculated as described earlier.

To determine if the dyes were adversely affecting the colonies, the number of dead workers in each colony at each reading date was recorded. The number of dead workers was summed after the last reading date and the percentage of dead workers (total number of dead workers/number of workers alive) determined. The percentage of dead workers was arcsine transformed. Colonies receiving dye were compared to those not receiving the dye (controls) by using general linear models (GLM) with a Dunnett's procedure (SAS Institute 1988, 1993) for the arcsine transformed variable.

Use of Marking Method in a Large Structure

On 18 March 1991, Pharaoh ants were monitored at the BOQ by placing two to six baits per room (one to two in the bedroom and living room windows, and two on bathroom floors where applicable) in preselected rooms in all wings. The bait consisted of 2 cc of natural peanut butter applied from a syringe onto an index card. Two hours later the baits were checked for the number of Pharaoh ants and removed. A bait card holding 6 cc of 2% Calco® red dye formulated in peanut butter was placed in room 162, the room with the highest Pharaoh ant count in the north wing. A similar bait card with 6 cc of 2% Calco® blue in peanut butter was placed in room 146, the room with the highest infestation in the south wing. Dye mixtures were left in place for 4 d, until 22 March 1991. On this day, the baits containing dyed peanut butter mixtures were removed and BOQ was baited again with plain peanut butter as on 18 March 1991 except that baits were not applied to the east wing because ants were not found there on 18 March 1991. Baits were also placed on the ground near the outside perimeter of the building starting from the entrance by the desk continuing around the north, west and south wings in a counter-clockwise direction. After 2 h, the plain peanut butter baits were collected. Ants found at the baits were tapped onto white paper, the paper folded over placed on a clipboard and the ants crushed with a heavy metal roller. If ants contained dye, the location where they were collected was recorded on a data sheet (drawn on a map for outside baits) and on the white paper on which they were crushed. The distance from the dyed bait at which ants containing dye were found (=dispersal distance) was determined and noted. The percent of workers containing dye on each card was determined in the laboratory the next day.

The procedures of 22 March 1991 were repeated on 29 March 1991 (dyed baits had been removed for 7 days) with a few exceptions. Only the lounge, soda mess (vending machine area) and rooms 228 and 229 were baited in the west wing. Also, baits found with ants were picked up and placed in a ziploc bag and the location marked on the bag. The bags were placed in a cooler with ice and then placed in a freezer in the laboratory. The locations where ants were collected, percentage of ants containing dye and dispersal distance were determined in the laboratory.

Dispersal distance was calculated under several assumptions. It was assumed that ants foraged along the outside wall because previous work in the BOQ indicated the majority of foragers were found near walls, windows or pipes. When ants were found in rooms on the other side of the building from where the dyed baits were placed, it was assumed that they travelled along structural guidelines (Klotz and Reid 1992) following right angles because they would either be following pipes or beams. Sudd (1960) found Pharaoh ants using trails that followed edges of doors and floors. Also, it was assumed that the colony was near to the position where the dyed baits were placed, since these positions had the highest infestations in each wing on 16 March 1991. All dispersal distances were measured from the position of the dyed bait. Dispersal distance measurements were verified by using blueprints obtained from the public works department at the Jacksonville Naval Air Station.

Dispersal distance was calculated and foraging (number of ants at bait, percent marked and foraging distance) of ants outside to ants indoors was compared using general linear models (GLM) (SAS Institute 1992). The same variables were compared for the two dates using the same test.

Results and Discussion

Development of a Marking Method

The dyes tested did not adversely affect worker survival or repel workers from feeding. The percentage of dead workers in the control colonies was not significantly different from those in treated colonies, for ants receiving dyes in unprocessed peanut oil or peanut butter (Table 2-1).

None of the treatments differed significantly from one another for percentage of bait consumed (Table 2-2). Although either method would have yielded similar results, the peanut butter mixtures at 2% were chosen for field use because they were easier to dispense and the 2% dyes were easier to detect than the lower concentrations in crushed ants.

The dyes were present in the foragers for at least 37 days in the 2% dyes in unprocessed peanut oil (Fig. 2-1). Lower concentrations were not sampled at 37 days. However, at 28 days percentage of workers containing dye had dropped to 37% in the blue 0.25% treatment. This dye was very difficult to see at that date and concentration. In general, the higher concentrations of dyes were much easier to detect. Also queens' postpharyngeal glands were still marked at 37 days for the 2% dyes in unprocessed peanut oil (Fig. 2-2). Even though brood was not observed until day 4, most small and large larvae contained dye. The gasters of workers inside the nest were clearly engorged with dye. When these workers were dissected, it was apparent the dye was present in the crop and postpharyngeal gland. Some larvae and eggs from colonies fed 1 and 2% Calco® red were pink on day 11.

In the treatments formulated in peanut butter, dyes were present in foragers (Fig. 2-3a) and queens (Fig. 2-3b) at least 39 days. By day 39, color was difficult to detect in those colonies which had received only the high concentrations of dye (1 and 2%). Most small and large larvae were dyed on day 4. Also, brood tenders were observed with gasters engorged with dye. Dye was apparently stored in the same organs as described for the colonies fed the dyed, unprocessed peanut oil. On day 11, pink eggs and larvae were observed in colonies fed Calco® red in peanut butter.

Use of Marking Method in a Large Structure

Both marked and unmarked ants were found on the plain peanut butter bait cards collected both indoors and outdoors 4 days (22 March, Fig. 2-4) and 11 days after initial bait introduction (29 March, Fig. 2-5). In both the north and south wings, Pharaoh ant foraging was extensive. Both horizontal and vertical movement of foragers was detected. Marked foragers were found in rooms on the same side of the hall and across the hall from where the baits were placed (Figs. 2-4 & 2-5) as well as on the floor above. The estimated foraging territory (area where dyed ants were detected) increased on the second monitoring date in the north wing. Ants were found containing dye further south on the west side of the building.

The results presented hereafter shall only refer to baits containing marked ants. The mean (± standard deviation) dispersal distance (distance a dyed ant was found from the dyed bait card) for both dates combined was 16.2 ± 9.6 m. The maximum dispersal distance was 45 m. This was greater than that found by Sudd (1960), Peacock and Baxter (1949) and Ritter et al. (1977b). The longest Pharaoh ant trail that Sudd (1960) measured in houses in Nigeria was 9.5 m. Peacock and Baxter (1949) measured a 12.2 m trail in Scotland. Ritter et al. (1977b) mentions that Pharaoh ant trails are many meters long. The dispersal distances recorded at the BOQ indicated the ants did have the potential to distribute a substance from one wing to the other.

The mean number of ants per bait card (117.2 ± 96.1) indicated a severe infestation. A mean of 49.9 ± 40.8% of the foragers crushed on the paper contained dye. There was a very weak negative relationship between the percentage of the ants marked (y) and the distance in meters from the dyed bait (x) ($y=80.90-12.91*[\ln(x)]$, $r^2=0.14$). Percentage of workers found containing dye on 29 March was significantly less than those found containing dye on 22 March 1991 (Table 2-3).

Dispersal distance along the outdoor periphery was significantly longer than that occurring indoors (Table 2-4). However, the number of ants per card was higher indoors than outdoors indicating that more workers were foraging indoors than outdoors. A similar study conducted in quadraplex units (buildings consisting of four apartments) in September and October in north central Florida indicated a majority of the Pharaoh ant foraging occurred outdoors (Oi et al. 1994).

Because dyes used could be spread to other colonies through trophallaxis, it is uncertain if the foraging of one colony, several colonies or the degree of trophallaxis between colonies was measured. Because of the lack of aggression between colonies, these colonies could be considered one supercolony. A supercolony as defined by Holldobler and Wilson (1990) is a "unicolonial population, in which workers move freely from one nest to another, so that the entire population is a single colony" (p. 643). This study documented the spread of a bait through an area, regardless of whether one colony or several colonies fed on the bait. Less than 6 cc and 2 cc of peanut butter were spread throughout the north (412 m^2) and south wing (2787 m^2), respectively, indicating that the use of baits as a control for Pharaoh ants should be very efficient.

To better quantify foraging of Pharaoh ants, a dye should be used that would mark workers and could be observed without killing the ant; and it should not be transferred to other individuals through trophallaxis. A dye which marks fat bodies would achieve these results; however, such a dye has yet to be located. Also, offering the baits containing dye for a shorter period of time may ensure that foragers from only the closest colony would have time to find the bait and be marked. Such modification of our techniques may allow for the estimation of colony size using a mark-recapture method similar to that developed by Su and Scheffrahn (1988) for termites. The ability to estimate colony size would enhance greatly the development of more judicious and efficient methods of Pharaoh ant control.

Table 2-1. Mean ± SEM percentage of dead workers per dye treatment

Dye	% dye	Last reading date	% of dead workers in unprocessed peanut oil	 peanut butter[c]
Blue	0.25	28[a]	23.2 ± 5.4	NA
	0.50	28	21.6 ± 1.7	NA
	1.0	28	21.0 ± 5.8	14.8 ± 2.8
Red	0.25	28	13.3 ± 5.9	NA
	0.5	28	28.6 ± 3.3	NA
	1.0	28	17.8 ± 4.2	20.2 ± 0.1
Control	0	28	20.8 ± 2.4	NA
Blue	2	37[b]	11.1 ± 0.1	13.1 ± 2.4
Red	2	37	12.8 ± 5.3	13.7 ± 2.1
Control	0	37	23.4 ± 2.3	14.9 ± 1.5

Last reading date refers to dyed unprocessed peanut oil only.

[a] N=2. Treatments not significantly different from control using arcsine transformation, GLM and Dunnett's procedure (F = 1.13; df = 6,13, P = 0.43 [SAS Institute 1993]); untransformed data presented.

[b] N=2. Treatments not significantly different from control using arcsine transformation, GLM and Dunnett's procedure (F = 3.29; df = 2,5, P = 0.18 [SAS Institute 1993]); untransformed data presented.

[c] dyed treatments N=3, control N=2. Treatments not significantly different from control using arcsine transformation, GLM and Dunnett's procedure (F = 1.6; df = 4,13, P = 0.21 [SAS Institute 1993]); untransformed data presented. NA, not applicable.

Table 2-2. Mean percentage ± SEM of bait consumed for dyes formulated in unprocessed peanut oil and peanut butter

Dye	% dye	% bait consumed	
		unprocessed peanut oil[a]	peanut butter[b]
Blue	0.25	31.5 ± 1.5	NA
	0.50	16.5 ± 2.5	NA
	1.0	29.0 ± 8.0	12.4 ± 1.7
	2.0	15.0 ± 6.0	11.3 ± 2.1
Red	0.25	28.5 ± 8.5	NA
	0.5	13.0 ± 2.0	NA
	1.0	16.0 ± 4.0	7.9 ± 1.3
	2.0	8.0 ± 2.0	12.7 ± 3.7
Control	0	15.5 ± 5.5	9.1 ± 1.8

[a] Not significantly different using arcsine transformation and GLM ($F = 2.71$; df 8,17; $P = 0.08$; SAS Institute 1985); untransformed data presented.

[b] Not significantly different using arcsine transformation and GLM ($F = 0.72$; df 4,13; $P = 0.60$; SAS Institute 1985); untransformed data presented. NA, not applicable

Table 2-3. Mean percentage ± SEM of workers containing dye, number of ants per card, an foraging distance compared by date

	3/22/91	3/29/91
% of workers with dye[a]	74.7 ± 6.0	26.6 ± 5.5*
number ants/card[b]	98.8 ± 13.7	136.1 ± 18.2NS
dispersal distance (m)[c]	15.7 ± 1.7	16.7 ± 1.6NS

*significant ($P < 0.05$), NS=not significant.

[a] Percentages were arcsine transformed before GLM (F = 33.7; df = 1,64; P = 0.001) was performed. Untransformed percentages are presented.

[b] (F =2.7; df = 1,67; P = 0.11).

[c] (F = 0.16, df =1,67; P = 0.69).

Table 2-4. Mean percentage ± SEM of workers containing dye, number of ants per card, and foraging distance compared by indoor and outdoor environment (22 and 29 March data combined)

	Indoors	Outdoors
% of workers with dye[a]	49.9 ± 5.4	50.4 ± 14.1 NS
number ants/card[b]	128.4 ± 13.0	64.0 ± 18.4*
dispersal distance (m)[c]	14.8 ± 1.2	22.6 ± 2.7*

*significant, NS=not significant.

[a] Percentages were arcsine transformed before GLM (F=0.01, df=1,64, P = 0.94) was performed. Untransformed percentages are presented.

[b] (F = 4.7, df = 1,67; P = 0.03).

[c] (F = 7.11; df = 1,67; P = 0.01).

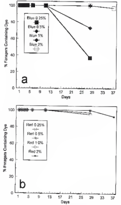

Figure 2-1. Percentage of Pharaoh ant foragers containing (a) blue or (b) red dye in unprocessed peanut oil.

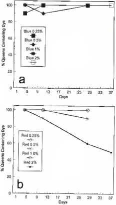

Figure 2-2. Percentage of Pharaoh ant queens containing (a) blue or (b) red dye in unprocessed peanut oil.

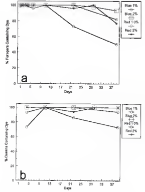

Figure 2-3   Percentage of Pharaoh ant (a) foragers or (b) queens containing blue or red dye in peanut butter.

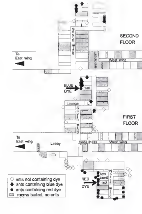

Figure 2-4. Results of Pharaoh ant baiting in the BOQ on 22 March, 4 days after initial baiting. Building plan is drawn to show location of the wings and the baits, and is not drawn to scale.

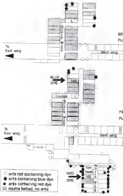

Figure 2-5. Results of Pharaoh ant baiting in the BOQ on 29 March, 11 days after initial baiting. Building plan is drawn to show location of the wings and the baits, and is not drawn to scale.

CHAPTER 3
SPATIAL DISTRIBUTION OF PHARAOH ANT IN SMALL BUILDINGS

Introduction

Although the place of origin of *Monomorium pharaonis* (L.) is disputed (Arnold 1916, Emery 1922, Bernard 1952, Wilson and Taylor 1967, Bolton 1987), most agree that it is of tropical origin. In temperate regions it survives in and is associated with heated structures with a few exceptions (Kohn and Vlcek 1984, 1986). In tropical areas, Pharaoh ants are associated with structures also, probably because of competition with other ants outside the structure (Edwards 1986). Reports suggest that Pharaoh ants nest outdoors in southern Florida (Creighton 1950, Krombein et al. 1979). In central Florida (Tampa), nests have been located in dead tree horns extending over structures (pers. obs.). To date, Pharaoh ants have not been found nesting outside of structures in northern Florida.

Like many other tramp ants, the Pharaoh ant possesses a reproductive strategy that does not require mating flights for sexual reproduction. They reproduce by budding. Budding occurs when workers remove the immatures with or without a queen to another nest site. Workers rear reproductives to perpetuate the colonies. Peacock et al. (1955) trapped budded colonies ranging in size from 92-4772 individuals. The largest number of workers trapped was 2000. The smallest colony with the simplest organization consisted of 100 workers and 103 eggs. A colony of 21 workers and 1 queen and a male pupa was the smallest laboratory colony maintained. Vail (unpublished) found that 5 workers and 30 eggs, 19 larvae, and 3 pupae can start a new colony. Colonies can be initiated with workers and brood; workers, brood, or queens, or less commonly, queens and brood (Sudd 1953)

Pharaoh ant nests are often cryptic, existing in cracks or crevices in walls, toilets, sinks, radiators, pipes, etc. in hotels, bakeries, cafeterias, apartments, and hospitals (Edwards 1986). Harris (1991) observed under laboratory conditions the effects environmental factors had on the distribution of Pharaoh ant nests. His investigations included the effects of previous occupation, wires, humidity, and moisture and food, on nest location.

Most studies of spatial distribution of Pharaoh ants in structures have pertained to foraging activity (Wilson and Booth 1981, Fowler et al. 1992, 1993, see Chapter 2). When nests were considered, they have been reported as trapped colony emigrations (Fowler 1993). In this study, nine buildings (floor space = 7.2 m^2) were constructed so walls were removable and attics were accessible. The location of a Pharaoh ant colony released into a building was monitored up to 30 wks to determine (a) the size and composition of satellite colonies, (b) Pharaoh ants nest sites indoors and outdoors, (c) if nests could be located by monitoring foraging activity, and (d) the effects of imported fire ants, *Solenopsis invicta* Buren, and other factors on nest composition and location.

Materials and Methods

Building specifications. Nine wooden buildings (2.4 m wide x 3.0 m long x 2.4 m high) were constructed on solid concrete slabs at the Medical and Veterinary Entomology Research Lab, USDA, ARS in Gainesville, FL. See Figure 3-1 for schematic of a building. Buildings were arranged in a 3 by 3 square with 15.2 m separating each building. T-111 siding (large wooden sheets with vertical grooves) was used as exterior walls. Fiberglass insulation (7.6 cm thick R-11) was used in the walls and attic. The ceiling consisted of sheet rock with 2 removable hatches (0.6 m x 0.9 m) allowing access to the attic crawl space. A ridge roof vent allowed for attic ventilation. Temperature was maintained by an air conditioner unit placed in the east wall and by a heater on the floor in the southeast corner. Interior walls consisted of wooden paneling attached with hinges on one side and turn buttons on one to three sides. Water was provided to ants in test tubes capped with cotton. Test tubes were placed in each corner of the building. One food cup, containing crickets and cotton wicks saturated with 25% honeywater (v:v), was tacked onto a stud around 61 - 76 cm above floor level on each wall.

Temperature was monitored in two ways: (1) instantaneously in 8 buildings using temperature and humidity recorders and (2) continuously in the central building using a CR10 datalogger (Campbell Scientific, Inc., Logan, Utah). In all buildings, 4 thermocouples were placed in all 4 walls and attic for a total of 20 thermocouples. Also, a hygrothermograph placed on a bucket painted with Teflon® in the southwest corner of each building recorded air temperature and humidity.

Colony introduction. Colonies to be introduced into the buildings were starved for 24 h and then dissected into three groups: brood (immature stages), workers, and female reproductives (mated and unmated grouped together). Each group was weighed. Queens and females were also counted. Colonies were starved prior to dissection so worker weight would be more uniform. It was assumed workers would have "dumped" their foraging load within 24 h. To determine total worker number, groups of ten were weighed from each colony to obtain an average worker weight. Total weights of workers were then divided by the average weight to obtain an approximate worker number.

Two weeks before colonies were introduced into the buildings, Amdro® (hydramethylnon [AI], American Cyanamid, Princeton, NJ) was applied at 1.3 lb/acre on grounds surrounding the buildings to reduce imported fire ant, *Solenopsis invicta* Buren, populations. One week later, the ground along each side of each building was treated with 1 tablespoon Amdro®. Several days before the first colonies were introduced, Maxforce® Pharaoh Ant Killer II bait stations (hydramethlnon, Clorox, Oakland, CA) were placed around buildings with fire ant activity and near active fire ant mounds on the grounds surrounding the buildings. These baits were removed prior to M. pharaonis introduction. Also, throughout the study, when S. invicta invaded the buildings, their mounds were located and removed by digging them up and placing them into a bucket where they were drowned with soapy water.

On September 20, two buildings were initially infested with Pharaoh ants and six others were infested 6 wk later. Colonies were introduced by placing a colony tray into the center of the structure floor. Food and water were removed from the tray and three to four scrapes through the Teflon® (Teflon® 30-B, Dupont Polymers, Wilmington, DE) were made on all sides of the tray to allow the ants to escape. Behavior of the ants as they left the tray and any interactions with other ant species were noted. Food (crickets and honeywater wicks) was provided twice a week in the feed cups. Water tubes were replaced as needed. Number and species of ants at food cups and water tubes were noted several times a week. Also, observations on ant presence and behavior on the outside of the structure were recorded.

Building inspection. Buildings were inspected to locate nests in the buildings once every 2 wk for 2 wk (bldgs. 1,3,6,7,8, and 9) or 8 wk (building 1 and 2) and thereafter once every 4 wk (all buildings). Attics were examined first by searching the interior of the roof with a flashlight. Insulation in the ceiling was systematically lifted from west to east. If a nest was located, several colony parameters were noted: number of workers and queens (including female alates), brood rating (Vail and Williams 1995), and presence of sex brood. Location of the nest (x and y coordinates) and the surface the nest rested on was also recorded. Temperature and humidity probes were placed near the nest, but not within the nest. Probes were not placed in the nest because we wanted to determine if nest site was chosen because of abiotic factors and not to determine if Pharaoh ants can regulate these factors. Attics were examined first to reduce the possibility of counting the same ants twice. These ants have been observed to move upward when disturbed (pers. obs.). The walls were searched next by releasing turn buttons and slowly swinging panels inward. Also, the aluminum and wooden doorplate was removed and searched for ants. Almost all possible nest sites were observable with this technique.

Foraging activity and nest location. To determine if ant activity at a bait indicated a nearby nest, index cards with about 1 ml of natural peanut butter was placed every

0.6 m along the edge of panelling, door or hatch in two buildings the first week in November and in all eight buildings the first week in February. Baits were left in place for 2-3 h and the number of Pharaoh ants at each card noted. Peanut butter on cards had been used successfully to monitor Pharaoh ant presence in structures (Willaims and Vail 1994, Oi et al. 1994). Location of nests were noted later in the day when the buildings were inspected.

Outdoor nesting. Pharaoh ants were observed to forage away from the buildings. To determine if ants were nesting away from the building, cards were placed around the outside of 3 buildings. One card with peanut butter was placed on the ground at each corner and center of wall. Cards were then placed every 1.5, 3.0, and 4.5 m away from those cards. Cards were checked at 0.5, 1, and 2 h after placement. If Pharaoh ants were found on a card, the foraging trail was followed to identify nest location.

Results and Discussion

S. invicta severely limited Pharaoh ant colonization and establishment in some of the buildings (3,6,8 and 9). The indoor presence of *S. invicta* at food cups ranged from 8 to 72% of the observation days (Table 3-1). On several occasions, *S. invicta* caused *M. pharaonis* to form small ephemeral nests on the panelling facing the interior of the room. These nests averaged (± SD) 47 ± 60 workers, and 0.4 ± 0.6 queens and female alates compared to the average nest found within the walls or attic with 1556 ± 1121 workers, 16.0 ± 1.8 queens and female alates, and 1.4 ± 1.2 g brood. Although the amount of brood present was not always noted, brood was found in 97% of the ephemeral nests. The small nests were usually gone within 2 to 3 d and probably coalesced to form a larger nest. On other occasions, *S. invicta* drove the Pharaoh ants from an entire wall as seen in the north wall of building one (compare Fig. 3.2 to 3.3).

The frequency distribution of workers, brood, and number of females are presented in Figs. 3-4, 3-5, and 3-6, respectively. Prior to release into the buildings, colonies ranged from 32000 to 74000 workers (mean and standard deviation of 56,287 ± 13617), 8.2 – 20 g brood (12.7 ± 4.3), and 236-1137 female alates and queens (617 ± 312). Nests observed in the buildings were more plentiful and much smaller than those released into the buildings indicating the colonies had budded. Budding could have been caused by overcrowding in the release tray and disturbance. Disturbance was present in several forms including the monitoring of the buildings and invasion by fire ants. While the effect of monitoring can not be determined, the effect of fire ants can The effect of fire ant frequency in entering buildings on the biomass (g), brood (g), number of females, and number of workers per colony (Figs. 3-7) and per building (Fig. 3-8) indicate the fire ants severely decreased the Pharaoh ant colony size. Fire ant presence in the buildings also decreased the mean number of Pharaoh ant nests per building (Fig. 3-9).

Fire ants also limited the duration of Pharaoh ant nests in the soil below the slab. Several Pharaoh ant nests, as indicated by the movement of queens, brood, and workers, were located under the slab of the buildings. *S. invicta* encountered the Pharaoh ants and several behavioral interactions occurred. Initially, there was gaster flagging by the Pharaoh ants and aggression. Battles ensued. Pharaoh ants were seen carrying *S. invicta* heads. Eventually, the Pharaoh ant colony moved indoors. The Pharaoh ants would, in some cases, continue to forage outdoors and avoid the fire ants or forage only indoors. Pharaoh ant colonies would persist outdoors under the slab until they encountered fire ants.

Although there was no similar pattern of nest distribution among the eight buildings, some generalities can be stated. Of the five walls (including the attic floor), more nests were found in the attic than in the other four walls ([*F* = 5.26; df = 4, 35; *P* = 0.002] Figure 3-10a). Pharaoh ants were discovered to have a definite vertical foraging element, unlike another tramp ant, *Tapinoma melanocephalum* (F.) (Fowler et al. 1992). Fire ants also displaced Pharaoh ants foraging vertically at bait stations (Chapter 5, Oi et al. 1994). Pharaoh ants may have moved upward to avoid competition or predation or to seek warmer temperatures, the percentage of nests in the attic did not increase significantly with attic temperature (Fig. 3-11a) or presence of fire ants (Fig. 3-11b).

A greater percentage of nests (51.4%) were found between the paper of the insulation and the paneling than the paper and sheetrock (24.7%), paper only (8.6%), insulation and stud (6.1), and others (Fig. 3-10b). Other locations included paper and stud; stud and stud; doorplate; outside; slab and stud; stud and sheetrock; insulation and paper; insulation; and insulation and inside of T-111 siding. These nest sites each accounted for <5% of the nests. Harris (1991) determined joints between studs, and under the entrance to the walls as common nest sites. His study did not include the use of insulation.

In large structures, Pharaoh ants are thought to saturate the habitat to pre-empt other ants from colonizing (Fowler 1993, Fowler et al. 1993). Our data appear to support this hypothesis. Ants did not choose to locate within one wall (with the exception of building 7 which only contained one small colony). Two weeks after the ants were released into the buildings, a mean of 7.5 ± 3.7 (SD) nests were dispersed throughout the building with a mean and a median of 3.5 and 4 out of 5 walls, respectively, containing nests.

Nests were not clustered around the reliable water or food sources we provided. Mean distance ± SEM from food and water sources were 1.8 ± 0.07 m (69.1 ± 2.6") and 2.4 ± 0.1 m (94.5 ± 3.9"), respectively. The furthest a nest could have been located from a food or water source (in the center of the ceiling) was about 3 m and 3.7 m, respectively. Studies by Harris (1991) indicated satellite colonies were relocated closer to water sources but food sources did not produce the same effect. Mother colonies did not relocate possibly indicating shelter was more important than water. In my study, fire ants caused nests to be located further from the food (P=0.056, Fig. 3-12a). Water tubes were located on the floor in each corner of the building. The Pharaoh ant has a high water requirement and is often seen imbibing water from raindrops and other sources (Sudd 1960). Increasing the exposure of Pharaoh ants to fire ants did not cause the Pharaoh ants to locate their nests further from the water tubes (Fig. 3-12b).

It appears that the Pharaoh ants will return to a previous nest site after a disturbance or they will occupy previously used sites. A nest site was designated as previously used if the center of a nest was within 4 cm of the center of a previously used nest site. The percentage of nest sites occupied previously ranged from 47 to 67. Even though fire ants reduced the percentage of nests sites occupied previously (Fig. 3-13), a mean ± SD for all buildings was 56.7 ± 6.5%. In laboratory choice tests, Pharaoh ants chose old or previously occupied nest boxes 80 to 100% of the time over new ones (Harris 1991). Some boxes had been left vacant 40 d and were still selected. Although the cause of this was not determined, they suspected semiochemicals were responsible. Semiochemicals known to be produced by Pharaoh ants include queen pheromones (Holldobler and Wust 1973; Edwards and Chambers 1984, Edwards 1987); monomorine I and III (Edwards and Pinniger 1978, Edwarnds and Chambers 1984) as trail pheromone constituents and probably alarm pheromones and defensive substances; faranal, the major constituent in the trail pheromone (Ritter et al. 1977a, Edwards and Chambers 1984); and a larval stomodeal and anal secretion that increases queen fecundity (Borgesen 1989, Borgesen and Jensen 1995); queen inhibiting pheromone (Petersen-Braun 1975); and mating pheromone(Holldobler and Wust 1973) Most of the pheromones are short-lived. Pharaoh ants may also perceive remains of food or refuse piles or cuticular hydrocarbons from the colony to determine this as a suitable nest location.

Foraging activity and nest location. Bait cards closest to food cups contained ants 60 ± 39.4% (mean ± SD) of the time. The bait card closest to the nest contained ants 52.3 ± 38.5% of the time. Nests were within 0.6 m of a card containing ants only 28.9 ± 15% of the time. Ants at bait cards more accurately reflect foraging trails to bait cards than nest location (Fig. 3-14).

Outdoor nesting. When cards containing peanut butter were placed around the perimeter of the buildings at 0, 1.5, 3.0, and 4.5 m away from the building, the farthest the Pharaoh ants were detected from the building was at 1.5 m. Some of these cards contained hundreds of workers. When foragers were followed from the cards, all foragers either returned to the building or to the slab under the building indicating that Pharaoh ants were not nesting away from the building.

Pharaoh ant colonies placed into small buildings immediately budded resulting in a mean of 7.5 colonies distributed over a mean of 3.5 walls. It is thought they distribute themselves throughout a structure to preempt other species. This did not prevent fire ants from entering the buildings and reducing the Pharaoh ant populations (colony size and number). Pharaoh ants were found to nest outdoors in Gainesville, FL; this had never been observed prior to this study. However, fire ants also limited the duration of Pharaoh ants in the soil. Fire ants are fierce competitors (Vinson 1994). Pharaoh ants apparently attempted to repel fire ants by spraying repellent while flagging their gaster. This was effective initially, but eventually failed. As fire ants become more common indoors, it is likely the interactions between these two species will become more common. Could fire ants control Pharaoh ants in structures? Because of the fire ants ability to sting and recruit effectively, it is unlikely their presence in structures would be tolerated.

Table 3-1. Presence of S. *invicta* at food cups in small buildings where Pharaoh ants had been released

Bldg	No. of observation days	% of observation days with S. *invicta* indoors	Periods of S. *invicta* invasion
1	78	8	12 Nov.; 8-17 Mar (5/5[a])
2	100	37	21 Sep-24 Oct (24/25); 2 Feb-10 Mar (13/16)
3	77	55	3 Nov-10 Dec (22/22); 21 Feb-14 Mar (10/18); 31 Mar-21 Apr (10/10)
4	99	12	4-12 Oct (8/8); 5 Dec-18 Dec (4/5)
6	74	57	7 Nov-8 Mar (52/54); 10-19 Apr (5/6)
7	80	36	17-13 Nov (4/5); 5 Dec-6 Jan (8/12); 18 Jan-2 Feb (7/9) 12 Feb (1/1); 21 Feb-10 Mar (9/9)
8	72	49	12 Nov-5 Dec (13/13); 4 Jan-3 Feb (11/14); 12 Feb-23 Feb (7/7); 6 Mar (1/1). 14-19 Apr (3/3)
9	74	72	3 Nov-20 Mar (49/50); 27 mar (1/1); 3-10 Apr (3/4)

[a] Numbers in parentheses indicate number of observation dates with S. *invicta*/number of observation dates.

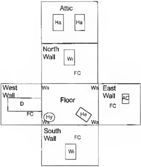

Figure 3-1. Building schematic with labels. Attic is lifted and placed above north wall. FC=food cup, Wa=water tube, AC= air conditioner, He=heater, D=door, Hy=hygrothermorgaph, Ha=hatch, and Wi=window

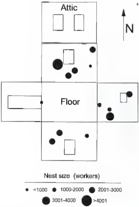

Figure 3-2. Pharaoh ant nest locations in building one (February 1995) Circles refer to number of workers in nest.

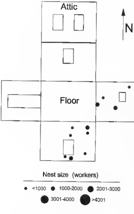

Figure 3-3. Pharaoh ant nest locations in building one (March 1995). Circles refer to number of workers in nest.

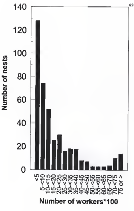

Figure 3-4. Frequency distribution of number of workers observed per nest.

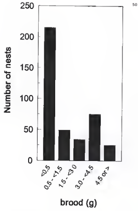

Figure 3-5. Frequency distribution for the amount of brood found per nest.

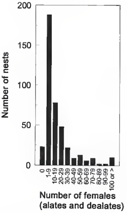

Figure 3-6. Frequency distribution for number of females (alates and dealates) found within each nest.

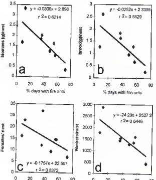

Figure 3-7. Effects of the percentage of observation days with fire ants on (a) mean biomass (g) per nest, (b) mean total grams of brood per nest, (c) mean total of females (alates and queens), and (d) mean total of workers per nest.

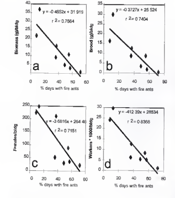

Figure 3-8. Effects of the percentage of observation days with fire ants on (a) mean biomass (g) per nest, (b) mean total grams of brood per nest, (c) mean total of females (alates and queens), and (d) mean total of workers per building.

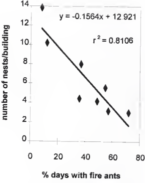

Figure 3-9. Effects of the percentage of observation days with fire ants on the number of nests per building.

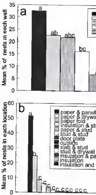

Figure 3-10. Mean percentage of nests in each (a) wall and (b) location. Letters indicate results of GLM and Ryan-Einot-Gabriel-Welsch Q test (P<0.05) (SAS Institute 1993).

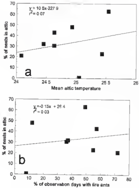

Figure 3-11. Effects of (a) mean attic temperature and (b) % of observations days with fire ants on the mean percentage of nests in the attic.

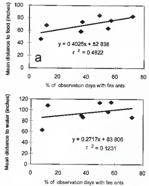

Figure 3-12. Effects of fire ants on the distance of nests to (a) food and (b) water.

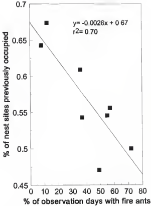

Figure 3-13. Effects of fire ants on the percentage of nests previously occupied.

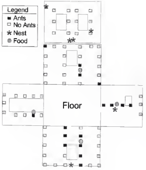

Figure 3-14. Results of baiting building four with peanut butter. Each square indicates the location of a monitoring card. Dark squares indicate the presence of ants and light squares the absence of ants. Ants at a bait card more accurately indicate foraging trails (food cups indicated by by oval) than nest location (nests indicated by asterisks).

CHAPTER 4

EFFECTS OF COLONY SIZE AND STARVATION ON FORAGING ACTIVITY OF THE PHARAOH ANT

Introduction

At least 8,800 species of ants are known to exist in the world today and some researchers estimate there are closer to 20,000 (Holldobler and Wilson 1990); however, colony size has only been estimated for about 150 species (Holldobler and Wilson 1990). Colony size is easily determined when nests are small and accessible (e.g. just below soil level, under pieces of bark, in decaying logs)(Wilson 1959). Even if the colony size is large and the nest is accessible, colonies can be excavated and counted or aliquots taken to estimate the number of workers (Tschinkel 1993). Also, mound size has been used to estimate number of workers (Macom 1994). Typically, the colony size of ants has been predicted using mark-recapture techniques (Golley and Gentry 1964, Erickson 1972, Gentry and Stiritz 1972, Rogers et al. 1972, Lewis et al. 1974, Kruk-de Bruin et al. 1977, Porter and Jorgensen 1981), but these are validated by excavating the nest upon termination of the study. This is not practical for a synanthropic pest; ant nests found inside buildings are cryptic and not easily accessible due to the location of the nest.

Pharaoh ant nests are unstructured and usually located in inaccessible or difficult to reach areas such as interior wall voids, areas under or behind window sills, toilets, sinks, switch plates, lights, voids in aluminum window and door frames. Although there are reports that suggest the Pharaoh ant nests outdoors in southern Florida (Creighton 1950, Krombein et al. 1979), in temperate regions it usually nests indoors [with an outdoor garbage dump noted as an exception (Kohn and Vlcek 1984, 1986)]. In north central Florida (Gainesville), Pharaoh ants were mainly observed indoors between the paper of insulation and the interior wall of panelling or drywall in small buildings. Some nests were found outdoors under the concrete slabs of the buildings (Chapter 3), but their association with the structure rendered them difficult to excavate. In most cases, Pharaoh ant nests are difficult to locate without causing damage to the structure.

Pharaoh ant colony sizes have been reported for emigrating colonies in buildings. Peacock et al. (1955) trapped founding (budding) colonies and found the smallest colony to contain around 100 individuals and the largest trapped to contain 4772 individuals. A migrating colony in a laboratory consisted of 47 queens, 693 workers, and 115 pieces of brood (O'Toole 1973). Approximately 350,000 workers and queens were removed from a single dwelling by Bellsvoya (1999). No doubt, these workers were from several colonies

It is the objective of this study to determine the foraging activity of Pharaoh ant colonies as affected by colony size and starvation. Results from this study will determine the feasibility of predicting Pharaoh ant colony size from the number of workers feeding at a bait.

Materials and Methods

Colonies. Three colonies, artificially split from their mother colonies on 10 June and 23 July 1992, were used in this experiment. It was assumed that colonies split on the same day would be nearly equal in size. The budded colonies were initiated with 3 queens, 0.2 g brood and 0.1 g (ca. 500) workers. Colonies were kept in plastic shoeboxes (31.5 x 16.5 x 8.5 cm) with sides coated with Teflon® 30-B (Dupont Polymers, Wilmington, DE) to prevent escape. A nest was initially constructed of a 30 ml plastic cup with a 0.6 cm-deep castone (Dentsply, York, PA) bottom. A 0.6 cm diameter hole was cut in the lid of the cup to allow foraging. Ants were fed twice a week with insects and honey agar in a paper cup. Water was available to the ants in a test tube capped with a cotton ball. On 12 October, colonies were transferred to a larger plastic tray (52 x 39.5 x 12 cm [Rubbermaid 400-5n, Plastic Corp., Detroit, MI]). Cup nests were then replaced with square petri dishes (9.5 x 9.5 x 9.5 cm) half filled with castone. Tops of cells were covered with yellow acetate to screen light, yet allow observation of the ants.

Colony dissection. To determine colony size, each colony was dissected into six parts: exterior workers (workers outside the nest), interior workers (workers inside the nest), queens (dealated female reproductives), alate female reproductives, males and brood. To dissect the colonies, exterior workers were removed from the tray by allowing them to climb onto a large index card and then tapping them into another container. To separate the interior workers from the brood, colonies were placed in a freezer until activity ceased. Cooling would cause workers which were carrying brood to drop the brood that would otherwise be difficult to separate from workers. As workers recovered from cooling they were allowed to climb onto an index card and tapped into another container. This process was repeated many times until the workers were separated from the brood. Queens and alates were retrieved from the colony using a pair of featherweight forceps. Once stages were separated, each group was weighed on an analytical balance. The total number of workers was determined by weighing five groups of ten workers from each group of exterior and interior workers to obtain an average worker weight for each worker type. Total weights of each worker type were then divided by the average weight to obtain an approximate worker number. Colonies were starved 24 h prior to dissection so worker weight would be more uniform. It was assumed workers would have "dumped" their foraging load within 24 h of obtaining it. Dissection was performed only once--after the 2-h observation and before the 96-h observation.

Starvation. Because of the time needed to dissect colonies (about 2 h per colony), the same colonies were used for the 2-h and 96-h observations on foraging activity. These times were chosen in an attempt to obtain extremes in foraging number. Two hours of starvation was sufficient to cause low level foraging. Haack (1987) observed that a very small percentage of larvae were cannibalized as an alternate food source when colonies were starved for up to 5 d. From his data, I determined that a greater percentage of exterior workers was likely to forage at a bait when colonies were starved for 4 d (96 h).

Foraging activity. A strip of blotter paper 2.5 x 23 cm was placed 7.6 cm from the end of the tray and 24 cm from the nest. Each blotter paper contained ca. 1 g of honey:cricket slurry (1:1 on weight basis) placed in a strip down the center of the paper. The honey:cricket mixture had been blended in a small blender to create a homogenous slurry. Williams (1990) evaluated many household food items for food preference and found honey to be the most attractive carbohydrate source. Insects, such as cockroaches and mealworms, were an acceptable protein and lipid source (Williams and Vail unpublished). Sudd (1962) described dead or dying insects on window sills as the main food source for Pharaoh ants in Nigeria. Pharaoh ants recruited differently to carbohydrates, oil, and protein (Haack 1987, Haack and Vinson 1990), so a bait containing all three food groups was chosen. Also, Edwards and Abraham (1990) indicated workers tend to alternate between carbohydrates and protein foods and Erpenbeck (1976) in Haack (1987), indicated foragers were probably carbohydrate and protein specialists. A bait containing all three food groups would therefore attract any forager.

The number of ants feeding on the bait was counted every 5 min for 30 min. Workers outside the nest that were not foraging were also counted.

General linear models (SAS Institute 1988) was used to determine the effects of colony size and starvation on Pharaoh ant foraging activity. Regressions of colony size on number of workers and percentage of workers at the bait; colony size on percentage of exterior workers; and number of workers per queen were performed using Microsoft Excel Analysis Toolpack (Microsoft Corp. 1994).

Results

Colony dissection results are listed in Table 4-1. Worker numbers ranged from ca. 8,000 to 26,000 per colony. Percentage of all workers which were exterior workers (y) increased as the number of all workers (x) increased (Fig. 4-1). The mean percentage which were exterior workers averaged 9.2 ± 4.7 (SD) per colony over all colony sizes.

The percentage of exterior workers ranged from 4.4 to 15.5. More workers per queen were found in the older, larger colonies (Fig. 4-2). A mean ± standard deviation of 260 ± 200 of workers per queen was found for all colonies combined.

Foraging activity, as measured by the number of workers feeding at the bait 30 min after bait introduction, was significantly affected by colony size (total number of workers) (F=13.76; df = 5,5; P< 0.006) and by length of starvation period (F=165.6, df=1,5; P<0.0001). Number of workers at the bait increased with colony size and starvation period (Fig. 4-3). The mean percentage of workers at the bait compared to all workers in a colony for all colony sizes was 1.3 ± 0.41 and 3.6 ± 1.12 for 2 h and 96 h of starvation, respectively. There was a negative relationship between percentage of foragers at the bait and colony size. After 96 h of starvation, 5.6% of the workers foraged for the 7500 ant colony as compared to 2.6% at the 26000 ant colony (Fig. 4-4). The long strip of bait prevented saturation of the bait by the workers; there was always room for more workers to feed. Percentage of foragers after 2 h of starvation was not correlated with colony size (Fig. 4-4).

When all ants outside the nest at 30 min. were added together for each colony, an average of 4.9 ± 1.7 and 7.7 ± 1.9 percent of the total ants at 2 h and 96 h starvation were considered exterior. These percentages were lower than the percentage of exterior workers recorded from colony dissection (4.4 - 15%).

Discussion

The percentages of workers feeding on the bait were lower than those found with other ant species (Table 4-2). These were calculated over long periods of time while the Pharaoh ant estimates were based on the number of ants feeding after 30 min. A higher percentage of foragers would be needed if the ants were at risk during foraging. The Pharaoh ants reared in the laboratory experienced very little risk when acquiring food, therefore a small percentage of foragers could perform this task. In the laboratory, food was provided on a given schedule (i.e, a predictable food source). There is also a lack of predators in laboratory rearing except for an occasional spider. Laboratory conditions should not differ from conditions in a structure, except where Solenopsis invicta is prevalent (Chapter 3).

The lower percentage of workers feeding in comparison to the exterior workers may indicate the workers were searching for another food source. Edwards and Abraham (1990) stated that Pharaoh ants will switch preference after feeding on the same food. In this study, ants were fed the same food they would receive if they were not participating in the study except it was offered in a different form.

Other reasons for increased number of nonforaging workers are plausible. Porter and Jorgensen (1991) found *Pogonomyrmex* workers outside the nest that were not foraging. These exterior workers' roles included forager, defender and refuse worker. The collective exploratory behavior of Pharaoh ants recorded by Fourcassie and Deneubourg (1994) was similar to the response seen in my study to a food source. They observed starved (9 - 10 d) and fed artificially created colonies for their exploratory response to a marked (previously exposed to Pharaoh ants) and an unmarked area. A minimal response occurred with fed ants, but a dramatic outpouring of ants occurred in the starved colonies - one colony almost entirely emptied when exposed to an unmarked area. A network of trails led to quick domination of a new area and they hypothesized the network would allow a returning forager to rapidly recruit to a food source such as an insect. Because Pharaoh ants do not retrieve prey, but dismember it, a quick response is needed to prevent other species from finding the prey insect. A network of trails was not apparent in this study, probably because the ants followed the edges of the tray (structural or guideline orientation (Klotz and Reid 1992)). Because Pharaoh ants reproduce by budding, these workers may have been searching for another nest site. In hospitals in Brazil, Pharaoh ants dominated baits and space throughout the institutions (Fowler 1993, Fowler et al. 1993). The spatial domination of an area by Pharaoh ants was explained as an attempt to prevent other species from occupying the area. Therefore, it is also likely that the exterior Pharaoh ant workers that were not foraging were exploring the area in an attempt to saturate the environment if suitable nesting sites were found.

Because colonies were starved for 24 h prior to dissection, the percentage of exterior workers should be between the percentages for 2 and 96 h starvation. Some percentages of exterior workers were greater than those starved for 96 h. The greater percentage of exterior workers found during dissection of the colonies was probably due to disturbance of the colony during this procedure.

Mirenda and Vinson (1981) did not account for workers outside the nest that were not foraging, yet they also observed a decrease in the percentage of workers foraging as colony size increased. In a colony of 2541 Solenopsis invicta workers, 62% of the workers were considered foragers and in a colony of 28,827 only 16% of the workers were foraging.

Other authors have investigated the effects of starvation on recruitment and they also found an increase in the number of recruits with starvation (Wilson 1962, Wallis 1962). It has been suggested that starvation lowers the threshold for the decision to forage.

Many factors can affect the foraging decisions of ants: foragers' physical caste (Pharaoh ants are monomorphic), age, prior experience, trip distance, thermal stress, resource quality and the colony's nutritional status (Traniello 1989). Not only is the colony's nutritional status important, but the stages present in the colony also affect foraging effort. Worker larvae are chief consumers of protein; but sexual larvae and queens require large amounts of protein and lipid (Stradling 1978).

Pharaoh ants' foraging decisions are affected by colony size, starvation levels, previous exposure to a bait (Edwards and Abraham 1990), competition with other ant species (Chapter 3), temperature, type of food and percentage of larvae in colony (Haack 1987), stage of colony (reproductive, founding or ergonomic [Oster and Wilson 1978]), and distance from food (Chapter 2).

It is unlikely that population size could be predicted in the field from foragers at a bait. If, for example, 300 ants were counted at a bait in a field situation this could relate to a small colony with about 500 workers under starved conditions or a large colony (about 25,000 workers) under satiated conditions. However, if starvation levels of Pharaoh ants in a structure were reduced by saturating an environment with a previously-fed-upon food containing the three food types every 0.6 m in all directions; and if temperature was held constant (about 27° C), and if all predators were removed, it is possible the population of Pharaoh ants in a structure could be determined by using baits. Under these conditions, about 1.3 ± 0.4% (mean ± SD) of the population should be found feeding on the baits.

Table 4-1 Results of colony dissection

Colony	exterior workers			interior workers			total workers	queens		female alates		brood
	mg	X mg/ind.	#	mg	X mg/ind.	#	#	mg	#	mg	#	g
1	1.2	0.23	350	1389.3	0.18	7567	7917	131.3	85	77.3	49	4.9
2	147.5	0.21	702	1737.8	0.20	8776	9479	157.0	101	23.9	16	3.[illegible]
3	236.0	0.20	1174	2395.1	0.16	13441	14615	156.1	116	110.6	81	4.3
4	367.6	0.16	2240	2247.1	0.17	13456	15696	50.4	28	48.2	27	2.8
5	650.7	0.19	3491	3507.4	0.17	21463	23954	39.2	61	39.2	25	4.5
6	650.8	0.16	3982	3550.0	0.16	21646	25629	27.7	51	27.7	17	3.3

Table 4-2. Percent of colony foraging for different ant species (modified from Porter & Jorgensen 1981)

Species	% of colony foraging	citation
Monomorium pharaonis	0.7 - 5.6	this study
Solenopsis invicta	16 - 62	Mirenda and Vinson 1981
Pogonomyrmex owyheei	5.8 - 9.1	Porter and Jorgensen 1981
P. badius	10	Golley and Gentry 1964
	10 - 15	Gentry and Stiritz 1972
P. californicus	11.5	Erickson 1972
P. occidentalis	10	Rogers et al. 1972
Atta cephalotes	6.1	Lewis et al. 1974
Formica polyctena	24.8	Kruk-de Bruin et al. 1977

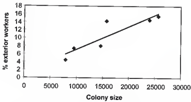

Figure 4-1. Percentage of workers outside the nest calculated from colony dissections Regression line is $y=5.7*10^{-4} + 1.4$ ($r^2=0.80$, standard error of slope $1.4x10^{-4}$, $P>t$ 0.016).

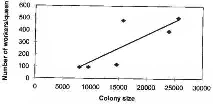

Figure 4.2. NUmber of workers per queen. Regression line is $y=0.022x - 82.15$ ($r2=0.66$, SE of slope 0.008, $P>t$ 0.048).

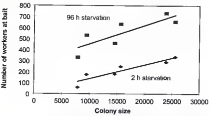

Figure 4-3. Number of workers at bait for each colony size (total number of workers) and two starvation periods. Regression lines for 2 and 96 h are $y=1.3*10^{-2}x + 10.9$ ($r^2=0.85$, standard error (SE) of slope $2.6*10^{-3}$, $P>t$ 0.009) and $y=1.7*10^{-2}x + 284.1$ ($r^2=0.59$, SE of slope $5.5*10^{-3}$, $P>t$ 0.039), respectively.

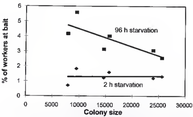

Figure 4-4. Percentage of workers at bait for each colony size (total number of workers) and two starvation periods. Regression lines for 2 and 96 h are $y=1.8*10^{-6}x + 1.3$ ($r^2=0.0012$, Standard error (SE) of slope $2.6*10^{-4}$, $P>t$ 0.95) and $y=1*10^{-4}x + 5.7$ ($r^2=0.64$, SE of slope $4.5*10^{-5}$, $P>t$ 0.05), respectively.

CHAPTER 5

EVALUATION OF PYRIPROXYFEN FOR CONTROL OF PHARAOH ANTS

Introduction

Pyriproxyfen, a juvenile hormone (JH) analog, (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine [McLaughlin Gormley King, Minneapolis, MN]), has been evaluated against urban insect pests such as cockroaches (Kawada et al. 1989, Ross and Cochran 1990, Koehler and Patterson 1991, Ross and Cochran 1991), fleas (Palma and Meola 1990), termites (Su and Scheffrahn 1989), and pest ants (Glancey et al. 1990, Banks and Lofgren 1991, Reimer et al. 1991, Vail and Williams 1995). In most cases, it was found to be an effective control agent. Shifts in caste (Su and Scheffrahn 1989, Glancey et al. 1990, Banks and Lofgren 1991, Vail and Williams 1995), mortality of immatures (Glancey et al. 1990, Banks and Lofgren 1991, Vail and Williams 1995), cessation of or decrease in oviposition (Glancey et al. 1990, Banks and Lofgren 1991, Reimer et al. 1991, Vail and Williams 1995), and toxicity to adults (Vail and Williams 1995) were noted in social insects exposed to pyriproxyfen in baits.

Pharaoh ants, *Monomorium pharaonis* (L.), are ubiquitous urban pests found indoors and outdoors in warmer climates, but restricted to indoor occupation in cooler climates

(Edwards 1986). Baits containing insect growth regulators, IGRs, such as methoprene and fenoxycarb, have provided control of Pharaoh ants (Edwards 1985, Williams 1990, Wilson and Booth 1981, Williams and Vail 1993,1994). Laboratory tests with a pyriproxyfen bait have proven effective in controlling Pharaoh ants (Vail and Williams 1995). Despite their proven efficacy, no IGRs are currently commercially available for Pharaoh ant control in the United States.

When Pharaoh ant foraging occurs outdoors, placing insecticidal baits where ants are actively foraging on the outside of structures reduces application time, risk to the applicator, cost, and exposure of residents to insecticides (Oi et al. 1994). Placement of baits containing metabolic inhibitors solely around the outside perimeter of structures has effectively controlled Pharaoh ants in Texas (Haack 1991) and Florida (Oi et al. 1994). The objectives of this study were to evaluate a peanut oil on corn grit formulation of pyriproxyfen for Pharaoh ant control when placed exclusively around the outside perimeter of a large structure. Also, laboratory colonies were subjected to the same baits to evaluate the efficacy of the material applied in the field.

Materials and Methods

Two concentrations (0.5 and 1%) of pyriproxyfen were formulated by weight in unprocessed peanut oil. Calco® red and blue dyes (2% by weight, Pylam Products Co., Inc., Garden City, NY) were added to the 0.5 and 1% solutions, respectively, to act as markers. This pyriproxyfen-oil-dye solution was applied to a pregel defatted corn grit carrier (Illinois Cereal Mills, Inc., Paris, IL) to compose 30% of the bait by weight; a control bait (0% pyriproxyfen) was formulated using unprocessed peanut oil. Each treatment was placed in a separate bait station using 1 g of 1% pyriproxyfen and the control bait and 2 g of 0.5% pyriproxyfen bait. The bait stations consisted of a petri dish (50 x 9 mm) with nine small holes in the lid that allowed Pharaoh ant entry, but prevented bait from falling out. In addition, the top and bottom of the petri dish were sealed together with acetone to seal the dish and prevent bait spillage.

Field--Large structure. A natural infestation of Pharaoh ants located at a military bachelor officer's quarters, Jacksonville, FL was used for this trial. This three-storied building consisted of three wings with a central area connecting all wings. The building resembled a motel with each room opening onto an outdoor concrete walkway. The outside wall of each room consisted of two to three glass windows reaching from ceiling to floor supported by aluminum framing. Most rooms consisted of a sink area, bedroom, and bathroom with shower and toilet (total floor area = 35 m^2)

On 26 September 1991, initial Pharaoh ant population estimates were made by placing index cards containing about 1 g of peanut butter at various locations throughout the building. After 2 h, ants on each card were counted and the cards removed. Then the east and west wings were treated with 84 of the 1 and 0.5% pyriproxyfen baits, respectively; the south wing and central area received 92 control baits. Two bait stations were placed on the outside of each room. Usually, one bait station was placed on the base of the middle window frame and one on the stucco that separated the rooms. Baits also were placed in the two end hallways surrounding the east and west wing to provide any ants foraging in these wings the opportunity to feed upon the bait.

Baits were applied once per week for 2 wk and removed 1 wk after application. Pharaoh ant outdoor populations were monitored using index cards containing 1g of peanut butter placed in the bait station locations 1 wk later and every 2 wk thereafter until 19 wk. In addition to outdoor monitoring, indoor monitoring was initiated 11 wk after initial baiting coinciding with the onset of cooler temperatures and low outdoor ant counts. Four corner rooms per floor per wing were used. Monitoring cards were placed in six locations: on the floor of the bathroom by the toilet, on the counter by the bathroom sink, on the floor by both sides of the bed and on the floor by the door and window. The cards were left in place for 2 to 3 h after which ant species found and number of individuals were recorded. Cards containing ants were placed in a Ziploc® bag and returned to the laboratory. There ants were crushed on a white piece of paper with a heavy metal roller to determine the presence of dye.

Descriptive statistics (mean and standard error) of the number of Pharaoh ants per card and percentage of dyed Pharaoh ants were calculated per room for each treatment.

Laboratory. Previously described baits and bait stations were given to laboratory colonies to evaluate the efficacy of the material applied in the field. Two bait stations were provided to each of three colonies and left in place for 1 wk at which time they were replaced with fresh bait and again removed after 1 wk. Following bait treatments, each colony was provided food cups twice a week containing house flies, hard boiled chicken egg and honey agar. Observations were taken once a week after the initial bait application. Observations recorded were worker number, queen number and brood rating. Brood (eggs, larvae and pupae) was rated by visually comparing photographs of known quantities of brood with the brood in a cell (Vail and Williams 1995).

Brood rating, and number of workers and queens were analyzed using general linear models and Ryan-Einot-Gabriel-Welsch Q Test (SAS Institute Inc. 1993) to determine differences in treatments.

Results

Field--Large structure. More workers were found per card in the control wing than the treated wings on every date except at 7 weeks after the first bait application and at wk 0 (Fig. 5-1). After wk 9, a mean ant count of >5 per card in baited areas was found only once, during wk 11. No ants were found indoors in the 1%-treated wing during any wk or in the 0.5%-treated wing after wk 17, although ants were found in the control areas during the entire study (Fig. 5-1).

Laboratory. Worker number gradually declined (Fig. 5-2). At 10 wk, the mean worker number for the control colonies was 14667 compared to 442 and 115 in the 0.5 and 1% pyriproxyfen-treated colonies, respectively. Two weeks after the first baiting and each week thereafter, there were significantly less workers in the treated colonies than in the control colonies (wk 2, $F = 10.04$, $df = 2,6$; $P = 0.0122$).

These laboratory tests simulated the reductions found in the field trials. In the lab, 8 and 9 wk were required to reduce worker populations by greater than 85% for the 1 and 0.5% pyriproxyfen-treated colonies, respectively. In the large structure, both concentrations reduced worker number by 85% (consistently) by wk 9; although 85% reduction in worker number had been achieved in the 0.5% treatment by wk 3, it did not remain so (Fig. 5-1).

The number of queens declined slowly in the treated colonies and were not significantly lower than the control until wk 10 (F = 6.35; df = 2,6; P =0.033, Fig. 5-2). Queens in treated colonies were seen wandering the trays starting around wk 5. The decline in queen number could be attributed to death by old age, starvation from lack of worker attention, or toxic effects due to the accumulation of pyriproxyfen.

The amount of brood declined rapidly following treatment (Fig. 5-2). Brood rating was significantly lower in the treated colonies than in the control colony for every week beginning 2 wk after the first baiting (Fig. 5.2, F = 124.0; df = 2,6; P = 0.0001). Pyriproxyfen apparently prevents development of immature workers.

Both the red and blue dye were apparent in colonies 1 wk after first bait treatment. Dyes were visible in the postpharyngeal gland of both queens and workers and in the crop of workers. Dye also was visible in most larvae indicating that pyriproxyfen was well distributed throughout the colonies. Dye was not apparent in any pupae examined.

Discussion

A large building with separate wings is not an ideal structure for evaluating baits for Pharaoh ant control. The mean foraging distance determined for Pharaoh ants in a large structure was 16.2 ± 9.6 m (mean ± SD), respectively (Vail and Williams 1994). Even though ants travel long distances and trophallaxis occurs between neighboring colonies (Edwards 1986), it is unlikely that ants at one end of a large structure would communicate with ants at another end. However, determination of the degree of communication between ant populations within a large structure is essential to evaluate baits for ant control in the field. Once separate populations have been delineated, this information could be used to improve statistical analysis. Dyes should also be used to determine if ants are foraging from one wing to the next.

Calco blue dye was an excellent marker to detect the distribution of the pyriproxyfen-oil bait. This dye was detected in the foraging population in the field for at least 15 wk. The blue dye was also detected in the postpharyngeal glands of the queens and workers for at least 18 wk under laboratory conditions. Dyes should be used to determine if ants are foraging from one treatment area to the next at a potential field trial site. If possible, those areas where contamination is detected should be excluded from evaluation

In the large building field trial, dye was well distributed (>80%) throughout foragers in both treated wings 3 wk after initial IGR applications (Table 5-1). However, 11% of the ants collected in the control wing contained dye at 3 wk after initial bait application (Table 5-1). Therefore, ants living in the control wing were contaminated with the IGR although not to the same extent as the ants in the treated wings. These dye data indicated there was no guarantee that collections of Pharaoh ants from each room where the large building trial was conducted represented a separate population. As a result, descriptive statistics were used with this study to prevent violating the assumption of independent samples used in ANOVA.

Pyriproxyfen baits formulated at 0.5 and 1% by weight in unprocessed peanut oil on a pregel defatted corn grit carrier reduced Pharaoh ant populations in the large building field trial (Fig. 5-1). Nine weeks after the first bait application, most workers in the pyriproxyfen treatments not affected directly by IGR toxicity were probably dying because they reached the end of their estimated maximum life span of 9-10 wk (Peacock and Baxter 1950). The elapsed time to reduction of worker numbers corresponded to our laboratory work (Fig. 5-2). In addition to bait contamination of the control wing by trophallaxis, the large building field trial could have been affected by removal of ants from the structure to determine dye longevity or by lowered outdoor temperatures with the coming of fall and winter. However, morning temperatures for sample dates on wk 11, 13, 15, 17, and 19 were 21.5, 16.5, 11.5, 20.5, and 15°C, respectively. The mean number of Pharaoh ants per card for the controls on wk 11 was 25; however, the number per card was much lower (5.2) on wk 17 when the temperature was similar to that of wk 11. Therefore, temperature reductions alone can not explain the decrease in ant abundance in the controls.

Despite the lack of conclusive results, application of baits containing unprocessed peanut oil on pregel defatted corn grits to the exterior of the building was effective (Fig. 5-1). Results indicate that the placement of the bait outdoors in the large building trial could have been more selective. The baits were placed approximately the same distance apart throughout the building. Baits placed on the first floor were often consumed by fire ants. Placing baits above ground where monitoring has indicated an infestation or where Pharaoh ants are currently foraging, may ensure that Pharaoh ants are the first foragers to locate and feed upon the bait (Oi et al. 1994).

Pyriproxyfen appears to be an excellent compound for controlling natural infestations of Pharaoh ants. Furthermore, IGRs such as pyriproxyfen may provide more long-term control of Pharaoh ants. Insect growth regulators were spread more extensively through neighboring colonies than metabolic inhibitors in laboratory evaluations (unpublished data). Metabolic inhibitors act more quickly, eliminating foragers before they can spread the toxicant to neighboring colonies. In the only field study comparing an IGR (fenoxycarb) to a metabolic inhibitor (sulfluramid, Raid Max Ant Bait, S.C. Johnson, Racine, WI) the IGR provided longer Pharaoh ant control (Williams and Vail 1994). Fenoxycarb baits formulated at 0.5% in peanut oil completely eliminated Pharaoh ants for 18 wks (wk 6 - 24), while Pharaoh ants were detected in rooms receiving sulfluramid bait 6 wk after ants were considered eliminated. Pharaoh ants may not be eliminated from a structure using a metabolic inhibitor if all colonies in a site are not located or if baits are not placed close enough to the nest site or foraging trails. Applying a bait containing an insect growth regulator 1 wk prior to a bait containing a metabolic inhibitor may provide both rapid kill and long-term control.

Table 5-1. Mean percent Pharaoh ant foragers containing dye in pyriproxyfen treatments from large building field trial

	Mean ± SD % foragers containing dye per		
Weeks[a]	control	0.5%	1%
3	11.3 ± 23.77	97.8 ± 3.52	84.0 ± 14.35
5	2.7 ± 7.56	52.2 ± 29.11	91.1 ± 14.21
7	7.3 ± 25.39	23.9 ± 27.78	90.8 ± 9.12
9	11.1 ± 33.33	0	85.4 ± 33.48
11	0	0	22.8 ± 35.20
13	0	0	12.9 ± 35.22
15	0.03 ± 0.13	0	18.7 ± 28.08
17	0	0	--[b]

[a] Weeks after first treatment
[b] No ants found

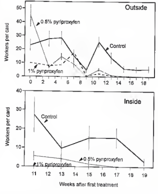

Figure 5-1. Mean number of Pharaoh ant workers per monitoring card placed outside or inside a military officer's quarters following treatment with an unprocessed peanut oil bait containing pyriproxyfen. No ants were found inside rooms treated with 1% pyriproxyfen (vertical bars

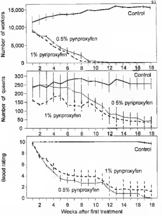

Figure 5-2. Mean number of workers, number of queens, and brood rating of Pharaoh ant colonies exposed to peanut oil baits containing pyriproxyfen (vertical bars indicate 1 standard error of the mean)

CHAPTER 6
CONCLUSIONS

Dispersal distances determined (mean = 16.2 m and maximum = 45 m) could indicate the foraging of one colony, several colonies or the degree of trophallaxis between colonies. Either way, this study documented the spread of a bait through an area, regardless of whether one colony or several colonies fed on the bait. Less than 6 cc and 2 cc of peanut butter was spread throughout the north and south wing, respectively, indicating that the use of baits as a control for Pharaoh ants should be very efficient.

In Gainesville, FL, Pharaoh ants were observed to nest outdoors in close association with a structure; this had never been recorded for this area. The establishment of fire ants in the Gainesville area may have limited the success of outdoor nesting by Pharaoh ants. Outdoor nesting and foraging further implicates the need for baiting outdoors.

The majority of Pharaoh ant nests in the attic could have implications for pest control. Placing baits where foragers are most active should provide the best control. However, if baits fail, attics, which are more easily accessed than walls, can be observed for ant activity and nests treated.

The presence of nests between the paper of insulation and panelling may indicate a need for a tight living space. Almost all nest were located in tight spaces where it would be easier for ants to regulate temperature and humidity. Future research will determine if these ants prefer a given nest space.

Foraging activity at food or water did not always indicate a nearby nest. This was proven in two ways. Ants were found feeding on bait cards placed at 0.6m along the edges of panelling, doors, and hatches although nests were greater than 0.6 m away in 71% of the nest cards. Also, nests were not located in close proximity to the food or water sources provided. Fire ants also caused nests to be located further from food sources.

The imported fire ant, Solenopsis invicta Buren, was an aggressive species that preyed on and caused budding and movement of Pharaoh ants nests. Fire ants infestations in and invasions into structures are becoming more common, as will the interactions between the Pharaoh ant and fire ant. Further research is needed on the interaction between these two species.

Even with fire ants and other factors causing a disturbance, Pharaoh ants returned to previously used nests sites on average more than 50% of time. If nest sites are easily accessed, these should be treated with an insecticidal dust to prevent future colonies from infesting the site.

Pharaoh ant foraging activity increased with starvation and colony size. However, the percentage of foragers at baits decreased with colony size. Because starvation and colony size significantly affected the number of ants feeding at the bait, it is unlikely that population size could be predicted in the field from foragers at a bait. If, for example, 300 ants were counted at a bait in a field situation this could relate to a small colony with about 500 workers under starved conditions or a large colony (about 25000 workers) under satiated conditions. However, if starvation levels of Pharaoh ants in a structure were reduced by saturating an environment with a previously-fed-upon food and predators, such as the imported fire ant, were removed, it is possible the population of Pharaoh ants in a structure could be determined by using baits. Under these conditions, about 1.3 ± 0.4% (mean ± SD) of the population should be found feeding on the baits.

Pyriproxyfen, a juvenile hormone (JH) analog, applied to the exterior of a military bachelor officer's quarters controlled natural infestations of Pharaoh ants. The use of baits outdoors exploits the ants outdoor foraging behavior. Because of fire ant competition at ground level baits, placing baits above ground where monitoring has indicated an infestation or where Pharaoh ants are currently foraging, may better ensure Pharaoh ants are the first foragers to reach the bait. Furthermore, IGRs such as pyriproxyfen may provide more long-term control of Pharaoh ants. Pharaoh ants may not be eliminated from a structure using a metabolic inhibitor if all colonies in a site are not located or if baits are not placed close enough to the nest site or foraging trails. Applying a bait containing an insect growth regulator 1 wk prior to a metabolic inhibitor bait may provide both rapid kill and long-term control.

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BIOGRAPHICAL SKETCH

Karen M. Vail was born on March 10, 1963, in Plainfield, N.J. Her interest in the environment was stimulated by a sixth grade teacher, Mrs. Gottleib, at Franklin School, South Plainfield. She graduated high school in 1981 and began attending Cook College, the Agricultural and Environmental Sciences College of Rutgers University. During her undergraduate career, a letter from her mom contained a newspaper clipping that indicated that entomology was a job of the future. She scoffed at this idea. She continued her studies in plant science with an option in integrated pest management and was awarded a B.S. degree in 1985. Three months later she entered the Department of Entomology at Virginia Tech as a master's student. Upon completing her degree in March 1988, she enrolled in the Ph.D. program in entomology at Virginia Tech. However, she was swept off her feet by by a post-doc in the department, Pat Parkman, and followed him to Florida when he was offered a research associate position there. So much for "not changing her life for any man." She was employed by the USDA, ARS, Medical and Veterinary Research Laboratory in July 1988 and entered into the amazing world of ants. Stimulated by these intriguing creatures, she began her pursuit of a Ph.D. at the University of Florida in January, 1991. Two children and five years later she received her Doctor of Philosophy degree from the University of Florida and will be employed as an urban entomology extension specialist at the University of Tennessee, Knoxville. Oh yeah, in June, 1989, she married Pat Parkman at the site where they had first met--Virginia Tech.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David F. Williams, Chair
Assistant Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jon C. Allen
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May 1996

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